

CLAIMS

What is claimed is:

1. A method of producing a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising:
 - 5 (a) collecting avian gonadal cells comprising primordial germ cells from an avian embryo after formation of the primitive streak;
 - (b) depositing the avian gonadal cells in contact with a preconditioned feeder matrix; and
 - 10 (c) growing the avian gonadal cells on the preconditioned feeder matrix in the presence of media for a time sufficient to produce an avian cell culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype.
2. The method of claim 1, wherein the avian gonadal cells are collected from a stage 14 to stage 45 embryo.
- 15 3. The method of claim 2, wherein the avian gonadal cells are collected from a stage 15 to stage 31 embryo.
4. The method of claim 3, wherein the avian gonadal cells are collected from a stage 27 to stage 30 embryo.
5. The method of claim 1, wherein the avian gonadal cells are
20 deposited in contact with the preconditioned feeder matrix in a number ranging from about 10,000 to about 20,000 cells.
6. The method of claim 5, wherein about 20,000 avian gonadal cells are deposited in contact with the preconditioned feeder matrix.

7. The method of claim 1, wherein the preconditioned feeder matrix comprises fibroblast cells.

8. The method of claim 7, wherein fibroblast cells are present in the preconditioned feeder matrix in a number ranging from about 40,000 to about 60,000 cells.

9. The method of claim 8, wherein fibroblast cells are present in the preconditioned feeder matrix in a number ranging from about 50,000 to about 60,000 cells.

10. The method of claim 9, wherein fibroblast cells are present in the preconditioned feeder matrix in a number ranging from about 55,000 to about 60,000 cells.

11. The method of claim 10, wherein about 60,000 fibroblast cells are present in the preconditioned feeder matrix.

12. The method of claim 7, wherein the fibroblast cells are mouse fibroblast cells.

13. The method of claim 12, wherein mouse fibroblast cells form a mouse fibroblast feeder layer.

14. The method of claim 12, wherein the mouse fibroblast cells are mouse STO fibroblast cells.

15. The method of claim 1, wherein the undifferentiated avian cells are capable of maintaining the stem cell phenotype when grown on the preconditioned fibroblast feeder matrix in the presence of the media for at least three days.

16. The method of claim 1, wherein the avian embryo is a embryo selected from the group consisting of chicken, turkey, duck, goose, quail and pheasant embryo.

5 17. The method of claim 15, wherein the avian embryo is a chicken embryo.

18. The method of claim 1, wherein the media is a conditioned media.

19. A method of producing a sustained culture of undifferentiated avian cells expressing an embryonic stem cell phenotype, comprising:

- 10 (a) collecting avian gonadal cells comprising primordial germ cells from an avian embryo after formation of the primitive streak;
- (b) depositing the avian gonadal cells in contact with a preconditioned fibroblast feeder cell matrix; and
- (c) growing the avian gonadal cells on the feeder cell matrix in the presence of media for a time sufficient to produce an avian cell
- 15 culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype.

20. The method of claim 19, wherein the avian gonadal cells are collected from a stage 14 to stage 45 embryo.

20 21. The method of claim 20, wherein the avian gonadal cells are collected from a stage 15 to stage 31 embryo.

22. The method of claim 21, wherein the avian gonadal cells are collected from a stage 27 to stage 30 embryo.

23. The method of claim 19, wherein the avian gonadal cells are deposited in contact with the feeder cell matrix in a number ranging from about 10,000 to about 20,000 gonadal cells.

24. The method of claim 23, wherein about 20,000 avian gonadal
5 cells are deposited in contact with the feeder cell matrix.

25. The method of claim 19, wherein fibroblast cells are present in the fibroblast feeder matrix in a number ranging from about 40,000 to about 60,000 cells.

26. The method of claim 25, wherein fibroblast cells are present in
10 the fibroblast feeder matrix in a number ranging from about 50,000 to about 60,000 cells.

27. The method of claim 26, wherein fibroblast cells are present in the fibroblast feeder matrix in a number ranging from about 55,000 to about 60,000 cells.

28. The method of claim 27, wherein about 60,000 fibroblast cells are
15 present in the fibroblast feeder matrix.

29. The method of claim 19, wherein the fibroblast feeder matrix comprises mouse fibroblast cells.

30. The method of claim 29, wherein the mouse fibroblast cells are
20 mouse STO fibroblast cells.

31. The method of claim 19, wherein the undifferentiated avian cells are capable of maintaining the stem cell phenotype when grown on the fibroblast feeder matrix in the presence of the media for at least three days.

32. The method of claim 19, wherein the avian embryo is a embryo selected from the group consisting of chicken, turkey, duck, goose, quail and pheasant embryo.

5 33. The method of claim 32, wherein the avian embryo is a chicken embryo.

34. The method of claim 19, wherein the media is a conditioned media.

35. A method of producing a sustained culture of undifferentiated chicken cells expressing an embryonic stem cell phenotype, comprising:
10 (a) collecting gonadal cells from a stage 27 to stage 30 chicken embryo;
(b) depositing the gonadal cells in contact with a preconditioned mouse fibroblast feeder cell layer;
(c) growing the gonadal cells on the preconditioned mouse fibroblast
15 feeder cell layer in the presence of a conditioned media for a time sufficient to produce a sustained avian cell culture, the sustained avian cell culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype.

20 36. The method of claim 35, wherein the gonadal cells are deposited in contact with the preconditioned mouse feeder cell layer in a number ranging from about 10,000 to about 20,000 gonadal cells.

37. The method of claim 36, wherein about 20,000 gonadal cells are deposited in contact with the preconditioned mouse feeder cell layer.

38. The method of claim 35, wherein fibroblast cells are present in the preconditioned mouse fibroblast feeder layer in a number ranging from about 40,000 to about 60,000 cells.

5 39. The method of claim 38, wherein fibroblast cells are present in the preconditioned mouse fibroblast feeder layer in a number ranging from about 50,000 to about 60,000 cells.

40. The method of claim 39, wherein fibroblast cells are present in the preconditioned mouse fibroblast feeder layer in a number ranging from about 55,000 to about 60,000 cells.

10 41. The method of claim 40, wherein about 60,000 fibroblast cells are present in the preconditioned mouse fibroblast feeder layer.

42. The method of claim 35, wherein the preconditioned mouse fibroblast feeder cell layer is a mouse STO fibroblast feeder cell layer.

15 43. The method of claim 35, wherein the undifferentiated avian cells are capable of maintaining the stem cell phenotype when grown on the preconditioned mouse fibroblast feeder layer in the presence of the media for at least three days.

Abstract of the Disclosure

- 5 A method of producing undifferentiated avian cells expressing an embryonic stem cell phenotype. The method includes the steps of collecting avian gonadal cells comprising primordial germ cells from an avian embryo after the formation of the primitive streak; depositing the avian gonadal cells in contact with a preconditioned feeder matrix; and growing the avian gonadal cells on the pre-conditioned feeder matrix in the presence of media for a time sufficient to produce an avian cell culture consisting essentially of undifferentiated avian cells expressing an embryonic stem cell phenotype.